Program Digest, 2019 IEEE International Ultrasonics Symposium (IUS)
Glasgow, Scotland, October 6-9, 2019

Intravascular Sonothrombolysis, *in vitro*, Using a Small Aperture, Forward-Viewing, Sub-Megahertz Transducer to Enhance tPA Treatment

Leela Goel[1,2], Huaiyu Wu[1], Howuk Kim[1], Bohua Zhang[1], Jinwook Kim[2], Paul Dayton[2], Zhen Xu[3], Xiaoning Jiang[1]

[1]The Department of Mechanical and Aerospace Engineering, North Carolina State University, Raleigh, NC, USA
[2]The Joint Department of Biomedical Engineering, The University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, NC, USA
[3]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA
xjiang5@ncsu.edu

***Abstract*— Catheter-based thrombolytic treatments with tissue plasminogen activator (tPA) have long treatment times (>15 hrs) and high risk of intracranial hemorrhage. Sonothrombolysis may improve patient outcomes while reducing the dose of tPA needed for treatment. We recently demonstrated a custom, forward-viewing intravascular (FVI) transducer with microbubbles (MBs) for *in vitro* sonothrombolysis. For clinical translation, we want to utilize this transducer to enhance tPA treatment. Therefore, the purpose of this study was to examine the thrombolytic outcomes of a forward-looking, IV transducer with tPA *in vitro*. Blood clots were treated for 30 minutes with either phosphate buffer saline (PBS), ultrasound (US) alone, tPA alone (1µg/ml), tPA + US, or MB + US (10^8 MB/ml). The percent clot lysis for the control group was 29 ± 4%, tPA alone was 29 ± 9%, and US alone was 25 ± 4%, with no statistically significant differences amongst these conditions. The percent clot lysis was 58 ± 10%, 59 ± 3% and 63 ± 5% for tPA + US and MB + US conditions respectively and both had statistically significantly more clot lysis than the control, US alone, or tPA alone groups. We have demonstrated that a forward-viewing intravascular transducer can be used to enhance tPA mediated sonothrombolysis and is comparable to microbubble mediated sonothrombolysis.**

***Keywords*— sonothrombolysis, intravascular sonothrombolysis, clot lysis**

I. Introduction

A. Background

One of the most common treatments for treating blood clots is the systemic administration of a thrombolytic agent, such as tissue plasminogen activator (tPA). This technique has long treatment times (>15 hours) and has the risk of patients developing intracranial hemorrhage [1]. There is a need for improved thrombolytic treatments which can both result in safe clot lysis while minimizing the risk of negative side effects in the patient.

Sonothrombolysis is the use of ultrasound to enhance clot lysis outcomes and has been shown to be clinically effective for transcranial applications when used mediated with tPA and with microbubbles (MBs) [2, 3]. The EKOS Endowave system is a high frequency, intravascular, side-viewing sonothrombolysis system which has been used for catheter-directed thrombolysis therapy. However, likely due to its low power and indirect insonation of the clot, there is little evidence that this intravascular system improves clot lysis outcomes compared to conventional catheter directed therapies [4].

Previous work from our group has shown that a sub-megahertz, forward-viewing intravascular (FVI) transducer can cause substantial clot lysis *in vitro* using MBs and no thrombolytic agent [5, 6, 7]. However, to assess the clinical translation of our device, we want to utilize this transducer to enhance tPA treatment.

B. Hypothesis and Purpose

The purpose of this study is to examine the thrombolytic outcomes of a forward-viewing intravascular transducer for tPA mediated sonothrombolysis compared to tPA alone and MB mediated sonothrombolysis.

We hypothesize that tPA mediated FVI sonothrombolysis will have higher clot lysis results than tPA treatment alone and will be comparable to MB mediated FVI sonothrombolysis.

II. Methods

A. Blood Clot Formation

Blood clots were prepared as previously described [5]. Briefly, bovine blood (Densco Marketing, Inc., Woodstock, IL, USA) was mixed with a calcium chloride solution in a 10:1 ratio (eg 50 ml blood/ 5 ml calcium chloride). The blood mixture was transferred to centrifuge tubes and incubated at 37°C in a water bath. Clots were then stored at 4°C for at least 72 hours for stable clot formation. The final clot ages used were 3-7 days old, prepared to a final mass of 140 ± 20 mg.

B. Treatment Condition Preparation

Recombinant tissue plasminogen activator was reconstituted per the manufacturer's instructions (Cathflo Activase, Genentech USA, Inc), aliquoted, and stored at -20°C until use. The aliquoted tPA was then thawed and diluted to a final working concentration of 1.00 µg/ml for the tPA alone and tPA + US conditions.

Lipid-based decafluorobutane gas-filled microbubbles were prepared as described previously [5]. The final working concentration of MBs used in the MB + US condition was approximately 10^8 MB/ml.

Phosphate buffered saline was used for the control and US alone condition.

C. *in vitro Experimental Setup*

Blood clots were weighed before and after treatment, with the percent mass decrease used as the metric for clot lysis. Prepared clots were placed in a vessel mimicking tube and fixed in a 37°C water bath. The FVI transducer and treatment injection catheter were fixed together and inserted into the clot tube (Figure 1). A 3-axis motion stage was used to ensure the transducer face was less than 0.5 mm away from the clot surface at all times.

The five treatment conditions examined were control, US alone, tPA alone, tPA + US, and MB + US. For all conditions, the treatment solutions were injected into the clot tube at a rate of 0.100 ml/min via a syringe pump. The treatment duration was 30 minutes. In conditions with ultrasound, a 500 kHz excitation was applied with peak negative pressure of 0.6 MPa, duty cycle of 5.7%, and pulse duration of 5 ms. The ultrasound output was manually switched "on" for 2 minutes and "off" for 30 seconds (still with a 5.7% duty cycle) to allow for diffusion of the treatment conditions into the clot [5].

D. *Statistical Analysis*

One-way ANOVA and Tukey's HSD tests ($\alpha = 0.05$) were performed on the percent mass decrease to evaluate statistical significance.

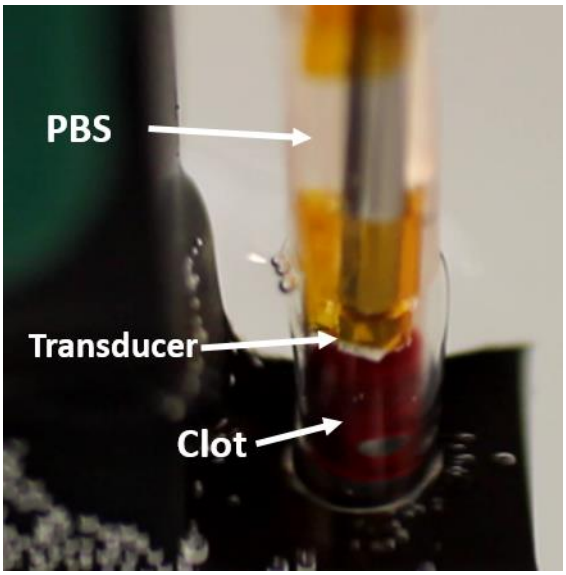

Figure 1. Experimental setup showing transducer and clot placement.

III. Results

The percent clot lysis for the control group was 29 ± 4%, tPA alone was 29 ± 9%, and US alone was 25 ± 4%, with no statistically significant differences amongst these conditions. The percent clot lysis was 58 ± 10%, 59 ± 3% and 63 ± 5% for tPA + US and MB + US conditions respectively and both had statistically significantly more clot lysis than the control, US alone, or tPA alone groups (Figure 2 and Figure 3). The tPA + US group also had similar clot lysis results as the MB + US group.

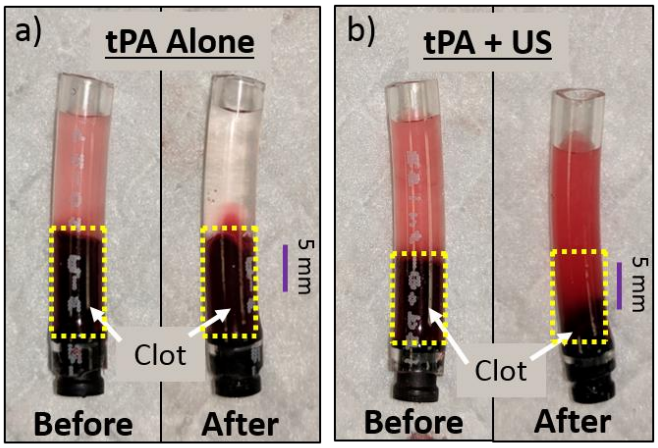

Figure 2. Representative images of clot lysis before and after treatment with a) tPA alone and b) tPA + Ultrasound.

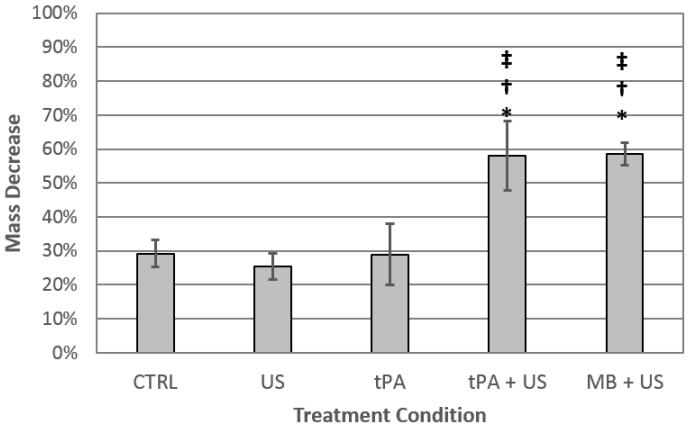

Figure 3. All Clot Lysis Conditions. The asterisk (*) indicate $p < 0.05$ compared to the control condition, the cross (†) indicates $p < 0.05$ compared to the ultrasound alone condition, and the double cross (‡) indicates $p < 0.05$ compared to the tPA alone condition.

IV. Discussion

This paper demonstrates that a forward-viewing intravascular transducer can be used for tPA mediated sonothrombolysis. FVI was able to almost double the amount of clot lysis in the tPA + US case compare to tPA alone. The lytic rates observed were similar to MB mediated sonothrombolysis. Additionally, these in vitro results suggest that FVI may be able to provide higher clot lysis results compared to the Ekos Endowave system, which was only able to induce clot lysis up to 16% in a similar recent *in vitro* study [6].

Future work should be done to explore the interaction and potentially synergistic effect of combined tPA + MB mediated sonothrombolysis with an FVI transducer. The safety of this technique should also be assessed.

V. Conclusion

FVI sonothrombolysis is a promising technique for tPA mediated sonothrombolysis, in vitro. Further work will be done to study the interaction of tPA with microbubbles and assess the safety of this technique.

Acknowledgments

The authors thank Brian Velasco for assistance in the microbubble formulation. This work was supported by NIH grant R01HL141967.

Declaration of Competing Interest

Xiaoning Jiang has a financial interest in SonoVascular, Inc. who licensed an intravascular sonothrombolysis technology from NC State.

References

[1] L. Viegas, E. Stolz, P. Canhao and J. Ferro, "Systemic Thrombolysis for Cerebral Venous and Dural Sinus Thrombosis: A Systematic Review," *Cerebrovascular Diseases,* vol. 31, pp. 43-50, 2013.

[2] G. Tsivgoulis, W. Culp and A. Alexandrov, "Ultrasound enhanced thrombolysis in acute arterial ischemia," *Ultrasonics,* vol. 48, pp. 303-311, 2008.

[3] C. A. Molina, M. Ribo, M. Rubiera, J. Montaner, S. E. R. Delgado-Mederos, J. F. Arenillas, R. Huertas, F. Purroy, P. Delgado and J. and Alvarez-Sabin, "Microbubble administration accelerates clot lysis during continuous 2-MHz ultrasound monitoring in stroke patients treated with intravenous tissue plasminogen activator," *Stroke,* vol. 37, no. 2, pp. 425-429, 2006.

[4] Y. Shi, W. C. L. Shi and J. Gu, "A systematic review of ultrasound-accelerated catheter directed thrombolysis in the treatment of deep vein thrombosis," *Journal of Thrombosis and Thrombolysis,* vol. 45, no. 440-451, 2018.

[5] J. Kim, B. Lindsey, W. Chang, X. Dai, J. Stavas, P. Dayton and X. Jiang, "Intravascular forward-looking ultrasound transducers for microbubble-mediated sonothrombolysis.," *Scientific Reports,* vol. 7, pp. 1-10, 2017.

[6] J. Kim, B. Lindsey, P. Dayton, W. Chang, H. Wu and X. Jiang, "Development of Forward-Looking Ultrasound Transducers for Microbubble-Aided Intravascular Ultrasound-Enhanced Thrombolysis," in *IEEE International Ultrasonics Symposium*, Washington DC, 2017.

[7] B. Zhang, H. Kim, H. Wu, Y. Gao and X. Jiang, "Sonothrombolysis with magnetic microbubbles under a rotational magnetic field," *Ultrasonics,* vol. 98, pp. 62-71, 2019.

[8] R. Engelberger, V. Schroeder, M. Nagler, R. Prince, D. Periard, D. Hayoz and N. Kucher, "Enhanced Thrombolysis by Ultrasound-Assisted Catheter-Directed Thrombolysis and Microbubbles in an In Vitro Model of Iliofemoral Deep Vein Thrombosis," *Coagulation and Fibrinolysis,* vol. 119, pp. 1094-1101, 2019.